

# Extrafloral nectaries alter arthropod community structure and mediate peach (*Prunus persica*) plant defense

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**Abstract.** We investigated the role of extrafloral nectaries (EFNs) in mediating plant defense for newly established peach (*Prunus persica*) trees. We used peaches of a single cultivar (“Lovell”) that varied with respect to EFN leaf phenotype (with or without EFNs) to determine if the EFNs affected the structure of the arthropod community colonizing newly planted seedlings. We also tested if the plants producing EFNs benefited from reduced herbivory or enhanced productivity. In the first year following planting, the young peach trees with EFNs were dominated by ants, and arthropod community diversity was lower than for trees without EFNs. The young trees with EFNs harbored fewer herbivores and experienced a twofold reduction in folivory compared to trees without EFNs. Productivity was also enhanced for the trees with EFNs, which attained significantly higher rates of trunk growth, greater terminal carbon composition, and a threefold increase in buds produced in subsequent years. In the second year of the field study, ants remained numerically dominant on trees with EFNs, but arthropod community diversity was higher than for trees without EFNs. An additional study revealed that folivory rates in May increased dramatically for trees with EFNs if ants were excluded from their canopies, indicating that ants have a protective function when the perennial trees produce new leaves. However, in later months, regardless of ants’ presence, the trees with EFNs suffered less folivory than trees lacking EFNs. The diversity and richness of the predator trophic group increased when ants were excluded from trees with EFNs, but overall community diversity (i.e., herbivores and predators combined) was not affected by the ants’ presence. Our research indicates that the EFNs play an important role in attracting predators that protect the trees from herbivores, and the EFN host-plant characteristic should be retained in future peach cultivar selections. Furthermore, peach production programs aimed at reducing insecticide inputs should seek to incorporate peach cultivars with EFNs, to optimize the potential for naturally occurring biological control agents to protect the trees from herbivores.

**Key words:** ants; biological control; community structure; diversity; extrafloral nectaries; Oedophrys hilleri; peach; plant defense; *Prunus persica*.

## INTRODUCTION

Extrafloral nectaries (EFNs) are secretory glands occurring on the petioles, stipules, and leaf margins of most peach (*Prunus persica* (L.) Batsch) cultivars (Gregory 1915, Okie 1998). The EFNs of some plants mediate plant defenses by attracting ants (Hymenoptera: Formicidae) and other predators or parasitoids (Lingren and Lukefahr 1977, Ruhren and Handel 1999, Cuautle and Rico-Gray 2003) that consume EFN exudates and protect the plants from herbivores (see Bentley 1977, Rogers 1985, and Bronstein 1998 for reviews). Recent findings indicate that the EFNs of *P. persica* augment ants that help protect the peach fruit from the oriental

fruit moth (*Grapholita molesta* (Busck); Mathews et al. 2007). The fact that EFN resources also are used by other predators (e.g., mites [Anystis, Anystidae; Pemberton 1993], chrysopids [Putman 1963], coccinellids [Pemberton and Vandenburg 1993]), and parasitic wasps (Lingren and Lukefahr 1977) could result in negative interactions (e.g., competition, intraguild predation) among ants and other taxa. Potentially, this could lead to a decoupling of the protective mechanism (Heil et al. 2004, Mody and Linsenmair 2004). Herbivores also exploit EFN resources (Wäckers et al. 2007). The response of the total arthropod community must therefore be considered before concluding that the EFNs have a positive impact in bolstering plant defenses. Our research evaluated the effects of EFNs on arthropod community structure and plant defense in peach.

The EFN secretions of *Prunus* spp. are composed of amino acids and sugars (Caldwell and Gerhardt 1986). The distribution and timing of EFN secretion in *Prunus*

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spp. are consistent with optimal defense theory, which suggests that young, nitrogen-rich leaves are more likely than old leaves to be protected chemically or via mutualisms with “bodyguards” (McKey 1974, Rhoades 1979). Both nitrogen concentration and EFN production in *P. persica* are higher in young leaves with less fiber than in older leaves (Yokoyama and Miller 1989). Leaf nitrogen concentration and EFN exudate volume of *P. persica* peak coincidentally in early spring when leaf-feeding herbivores may cause severe injury to trees (Putman 1963, Yokoyama and Miller 1989). By attracting bodyguards during this critical period, the EFNs could reduce deleterious effects to the plants.

In this study, we hypothesized that peach trees with EFNs harbor more ants than those without EFNs and, in turn, benefit from reduced folivory and enhanced productivity. We speculated that ants' predominance on trees with EFNs diminishes the diversity and richness of the overall arthropod community. We further hypothesized that removing the ant mutualists decouples the EFN-mediated defensive mechanism, leading to increased folivory for trees with EFNs.

#### MATERIALS AND METHODS

Field experiments that compared peach trees with and without EFNs were conducted in 2002 and 2003 at two sites (U.S. Department of Agriculture Appalachian Fruit Research Station, Kearneysville, West Virginia, and University of Maryland Western Maryland Research and Education Center, Keedysville, Maryland, USA). The two locations (situated 28 km apart) were similar in climate, topography (Hagerstown Silt-Loam soil; 3–8% slope with south-facing aspect), and surrounding habitat (unmanaged hedgerow ~10 m to the west, dirt road ~4 m to the north, and grass sod to the east and south). Treatments (EFNs present or absent) were arranged in a completely randomized design with four replicates (two per site). At each field site, replicates (0.25 ha each) were spaced 33 m apart and separated by a buffer hedge row of hybrid willow (*Salix* sp.) trees without EFN glands. A replicate consisted of two plots with each plot containing 30 trees (3-m spacing in five rows spaced 5 m apart) separated by a buffer hedgerow. *P. persica* has three leaf EFN phenotypes: globose (small, circular EFN glands), reniform (large, kidney-shaped EFN glands), and no glands (Okie 1998). EFN phenotype displays codominant inheritance, with heterozygotes producing trees with the globose type and homozygous alleles producing trees with reniform glands and no EFN glands (Connors 1922, Weber et al. 1997). Our studies used trees with reniform glands and trees with no EFN glands, produced by crossing plants of the cultivar ‘Lovell’ with different EFN traits. Plants were expected to differ only with respect to the EFN traits, and previous laboratory assays confirmed that the leaves with and without EFN glands were equally palatable to common herbivores (Mathews

2005). The 40 trees within a plot had the same leaf phenotype (reniform EFNs or no EFNs).

Dormant peach seedlings, produced from open-pollinated crosses of ‘Lovell’ parent material in a nursery seedling lot in 2001 (Adams County Nursery, Aspers, Pennsylvania, USA), were potted in March 2002 and held in the greenhouse at 18–21°C for 4 weeks to break dormancy. Trees (320 total) of a consistent size range (8–10 mm stem diameter, 60–65 cm height) were selected after verifying leaf EFN phenotype (160 trees with reniform glands, 160 trees without EFN glands). All trees were treated with avermectin (Agri-Mek 1.0 mL [active ingredient, AI]/L, 22 April; Syngenta, Greensboro, North Carolina, USA) to eliminate arthropods that may have infested them in the greenhouse and planted (26 April) in field plots that had been disked (19 April) to break up sod. Hybrid willow trees (1 m tall; Greenwood Nursery, McMinneville, Tennessee, USA) were planted into buffer rows separating the plots of each site (1-m spacing, 17 May). Mechanical cultivation to deter weed growth was performed between tree rows (11–12 June, 2–3 July, and 12–14 August 2002; 9–10 July and 4–5 August 2003), and a 0.5 m diameter area from the base of each peach tree was regularly hand-weeded from June to September (both years). Preemergent herbicides (oryzalin 0.55 kg [AI]/ha and oxyfluorfen 0.09 kg [AI]/ha) were applied under tree rows on 15 April 2003, and paraquat (0.12 kg [AI]/ha) was applied 14 May and 31 July 2003. Fungicide application was required both years to control heavy infestations of powdery mildew (*Sphaerotheca pannosa* (Wallroth ex Fr.) Lév) that can kill small peach trees (Rubigan 1.3 L[AI]/ha [Gowan, Yuma, Arizona, USA] and Penncozeb 6.7 kg[AI]/ha [United Phosphorus, King of Prussia, Pennsylvania, USA], 29 July 2002, West Virginia site only; myclobutanil 0.027 kg [AI]/ha, 18–23 June and 11–14 July 2003, both sites). Chlorpyrifos (5.7 L[AI]/ha) was applied to tree trunks by handgun sprayer in all plots for control of peachtree borers, *Synanthedon exitiosa* (Say), after completion of experiments in 2002 and 2003. No insecticides were applied during the field study months (May–September) of either year.

#### Extrafloral nectary effects

*Arthropod community structure.*—A two-year field study compared the abundance and diversity of herbivorous and predaceous arthropods associated with young peach trees of the two leaf EFN types. Predator and herbivore densities in the tree canopy were estimated twice monthly (24 and 31 May, 7 and 18 June, 3 and 16 July, 5 and 21 August 2002; 20 and 27 May, 11 and 26 June, 7 and 21 July, 14 and 28 August 2003) by limb jarring (Southwood 1966). Randomly selected trees (10 trees per plot in 2003, 4 trees per plot in 2002) were sampled between 07:00 and 11:00 hours. A randomly selected limb was tapped three times with a rubber hose while holding a 0.58-m<sup>2</sup> canvas tray underneath to collect dislodged arthropods. Dislodged arthropods

were identified on sight to order, family, or species, and replaced, so as not to disrupt the arthropod community. Individuals were tallied and grouped according to trophic level as “herbivores” or “predators” for data analysis. Actively foraging ants in the canopy of one randomly selected tree per treatment plot were collected on 21 August 2002 and 28 August 2003 and later identified by J. Sossa (Department of Systematic Biology, Ant Laboratory, Smithsonian Institution, Washington, D.C., USA) and S. Brady (Laboratory of Analytical Biology and Department of Entomology, Smithsonian Institution, Suitland, Maryland, USA).

The structure of the arthropod communities associated with the two tree types (with and without EFNs) was characterized using paired rank-abundance plots (Longino and Colwell 1997) and indices of diversity (Simpson 1949) and richness (Margalef 1958). Because it was not possible to resolve all taxa to species level in the field, community characterization was based on the total number of individuals in each taxon (family level for the Insecta, order level for the Arachnida) collected over the entire season. Richness ( $R$ ), diversity (Simpson's  $D$ ), and standard error (SE) were calculated for each treatment (with and without EFNs) and each year (2002 and 2003). Arthropod abundance (logarithmic scale) by taxon was ranked for trees with EFNs and plotted with the corresponding abundance (logarithmic scale) for trees without EFNs. The fixed effect of EFNs on the total number of arthropods by trophic level (herbivores, predators) was assessed by mixed-model analysis of variance (ANOVA) each year (SAS Institute 1999). The predominant herbivore and predator groups, *Oedophrys hilleri* (Faust) (Coleoptera: Curculionidae) and ants, respectively, were also analyzed. Temporal trends in arthropod abundance were assessed through independent analyses of data from each of the 16 sample periods after log transformation (SAS Institute 1999). The Bonferroni adjustment was used to correct for multiple comparisons.

**Folivory.**—Feeding assays in the laboratory showed that of the three leaf-feeding herbivores that regularly inhabited the peach tree canopies (*O. hilleri*, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and *Diabrotica undecimpunctata howardi* (Barber) (Coleoptera: Chrysomelidae)), only *O. hilleri* and *P. japonica* caused measurable foliage injury, and their feeding was easily separated in the field (Mathews 2005). A leaf preference test revealed no difference in feeding by *O. hilleri* and *P. japonica* adults between the two leaf types ( $N = 20$  leaves; percentage leaves with feeding during 72-h exposure, EFN glanded, 100%; glandless, 99%).

The level of folivory by *O. hilleri* and *P. japonica* was estimated for newly formed leaves on 10 randomly selected trees of each treatment plot. Sampling was limited to newly formed leaves on the distal portion (up to 5 cm from the tip) of the actively growing terminal, where glandular activity is concentrated (Yokoyama and Miller 1989). Younger leaves of a terminal are

commonly folded longitudinally. On trees with actively producing EFNs, the leaf margins of the young leaves stick together, impeding examination of the whole leaf. Therefore, the fifth distal leaf was used as the standard position for leaf samples of all trees. Five terminals of each sample tree were randomly selected, and the fifth distal leaf of each terminal was removed (16 July and 1 August 2002). The five leaves were examined visually for herbivory, caused by *O. hilleri* or *P. japonica*, and the percentage of injured leaves was recorded. To estimate the amount of extant leaf tissue available for photosynthesis, leaf surface area (square centimeters) was determined for the same five leaves per tree using a leaf area meter (LI-3100; LI-COR Environmental, Lincoln, Nebraska, USA). Separate mixed-model ANOVAs were performed within sample dates to test for an effect of EFNs on either folivory or leaf area (SAS Institute 1999). When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure ( $\alpha = 0.05$ ; SAS Institute 1999), and the Bonferroni adjustment was used to correct for multiple comparisons.

**Tree productivity.**—Three measures of tree productivity were examined to determine if EFN production was costly to the plant. Terminal carbon assimilation, an important indicator of seasonal productivity in terrestrial plants (Ehleringer 1991), was compared for trees with and without EFNs during the dormant period following the first year of growth (eight trees per treatment). On 1 March 2003 the distal 20 cm of three terminals per tree was removed from three random locations of a similar canopy height according to Ehleringer's (1991) procedures. After drying for 48 h at 55°C, samples were dipped in liquid N and ground to a fine powder (passing through a 40-mesh screen) in a stainless steel mill (LM-17-732, Wiley). The mill was cleaned with pressurized air between samples. The three pulverized samples from each tree were combined, and isotopic discrimination was performed for a 5-mg subsample ( $^{13}\text{C}$  molar abundance ratio,  $\Delta$ ; Isotope Services, Los Alamos, New Mexico, USA). The diameter of a tree's trunk correlates with aboveground biomass and is commonly used to predict potential fruit yield of deciduous fruit trees (Westwood and Roberts 1970). Trunk diameters (millimeters) were recorded for eight randomly selected trees per treatment during the dormant periods following the first two growing seasons. On 1 March and 1 October 2003 two perpendicular trunk diameters per tree were measured ~5 cm above the soil level using calipers, and the two measurements per tree were averaged. In the first year of fruit production (2004), bud production was evaluated (eight randomly selected trees per treatment). The number of flower buds formed on the distal portion (35 cm) of a randomly selected terminal was recorded for each tree (5 April). Data for the three productivity parameters were subjected to independent mixed-model ANOVA to test for an EFN effect.

### *The role of ants*

To determine if EFN-mediated plant protection is exclusive to ants, or if other predators play a protective role for *P. persica*, ant exclusion experiments were conducted on trees with and without the EFNs. An ant exclusion treatment was added to the EFN treatment structure (EFNs present, EFNs absent) in a completely randomized split-plot design (leaf EFN phenotype equals whole-plot factor; ant exclusion equals subplot factor). Sample trees (eight randomly selected, 3 April 2003) from each whole plot containing *P. persica* of the same EFN type were vigorously shaken to remove ants, and a band of masking tape (5 cm wide) was affixed to each trunk ~30 cm from the ground. The ant exclusion treatment (2 cm wide sticky barrier of tangle trap; Tanglefoot, Grand Rapids, Michigan, USA) was applied to one-half of the sample trees (four trees total). Trees receiving the exclusion treatment were inspected weekly, and tangle trap was reapplied as necessary to ensure effectiveness of the ant barrier. The ground under the sample trees' canopies was hand weeded weekly to prevent ants from crawling from ground vegetation to the canopies.

*Ants' impact on arthropod community structure.*—Arthropod abundance was estimated monthly between 07:00 and 11:00 on four trees per subplot (27 May, 26 June, 21 July, and 14 August 2003) by limb jarring. Dislodged arthropods were identified, tallied, and replaced so as not to disrupt the arthropod community. Arthropods were grouped according to trophic level as herbivores or predators for data analysis. The Simpson's diversity index and standard error (Simpson 1949) and Margalef's richness index (Margalef 1958) were calculated using the total number of individuals in each taxon collected over the entire season. Diversity and richness were calculated for the total arthropod community and the predator trophic level by treatment (with and without EFNs, with and without ant exclusion). The main effects of the EFN phenotype and ant exclusion treatments and the interactive effects were assessed for each sample date through independent mixed model ANOVAs (PROC MIXED, RANDOM replicate, replicate  $\times$  treatment, replicate  $\times$  treatment  $\times$  ants; SAS Institute 1999). The Bonferroni adjustment was used to correct for multiple comparisons.

*Ants' impact on folivory.*—Leaf herbivory was evaluated within 1–5 d after arthropod sampling (29 May, 27 June, 22 July, 19 August 2003). Five terminals per tree were randomly selected, the fifth distal leaf was removed, and the percentage of leaves with visible herbivory was recorded. Potential effects of EFNs, ant exclusion, and the interaction were assessed within each month through independent mixed-model ANOVAs (PROC MIXED, SAS Institute 1999) using the Bonferroni adjustment to correct for multiple comparisons. To test for an association between *O. hilleri* abundance and folivory, *O. hilleri* data (collected by limb jarring 27 May, 26 June, 21 July, and 14 August 2003) and leaf

damage estimates from corresponding dates were combined and subjected to correlation analysis by treatment (PROC CORR Spearman's, SAS Institute 1999).

## RESULTS

### *Extrafloral nectary effects*

*Arthropod community structure.*—Peach trees with EFNs supported nearly 6 $\times$  more individual arthropods than peach trees without EFNs in 2002 but only about 1.3 $\times$  more in 2003 due to a strong decline in ant numbers in 2003. Complete arthropod abundance data for 2002 and 2003 are provided in Appendix A. Except for the predator *Coccinella septempunctata* L., which occurred only on trees with EFNs, the same arthropod taxa were collected on the peach trees in the first year (2002), regardless of tree phenotype (Appendix A). Ants were numerically dominant (accounting for 92.8% of arthropods collected) on the trees with EFNs, which supported a less diverse and less evenly distributed arthropod community than the trees without EFNs in 2002 (Fig. 1, 2002). In the second year (2003) community diversity was higher for the trees with EFNs than those without EFNs, and richness was similar for the two tree types (Fig. 1, 2003). The ants *Formica nitidiventris* Emery and *Lasius neoniger* Emery were collected from trees of both leaf phenotypes. The weevil *Oedophrys hilleri* (Coleoptera: Curculionidae) was the dominant arthropod on trees lacking EFNs in both years.

The predator and herbivore trophic groups were significantly affected by the EFNs in both years (annual abundance; Appendix B). Trees with EFNs supported an average of 20 $\times$  more individual predators than those without EFNs in both years (LSD [least significant difference],  $P < 0.05$ ; Fig. 2a, b). Average annual ant abundance was significantly higher on trees with EFNs in both years (LSD,  $P < 0.05$ ; Fig. 2a, b). Average annual herbivore abundances were significantly lower on trees with EFNs than trees without EFNs in both years (LSD,  $P < 0.05$ ; Fig. 2a, b). Differences were more pronounced in the second year (2003) when average annual herbivore abundance for trees with EFNs was one-half that of trees without EFNs (Fig. 2b). Annual *O. hilleri* abundance was significantly lower on trees with EFNs in 2003 (LSD,  $P < 0.05$ ; Fig. 2b).

After ants had colonized the plots (~20 May) in both years, the peach trees with EFNs maintained significantly larger ant populations than the trees without EFNs for the rest of the peach growing season (Appendix C; Fig. 3a; LSD, Bonferroni,  $P < 0.05$ ). By contrast, the trees with EFNs had smaller herbivore populations than the trees without EFNs throughout the growing season (Appendix C; Fig. 3b; LSD, Bonferroni,  $P < 0.05$ ). However, differences in herbivore densities due to EFNs were statistically significant in less than one-half of the sample periods (Appendix C).

*Folivory.*—The leaf surface area did not differ significantly between trees with and without EFNs.

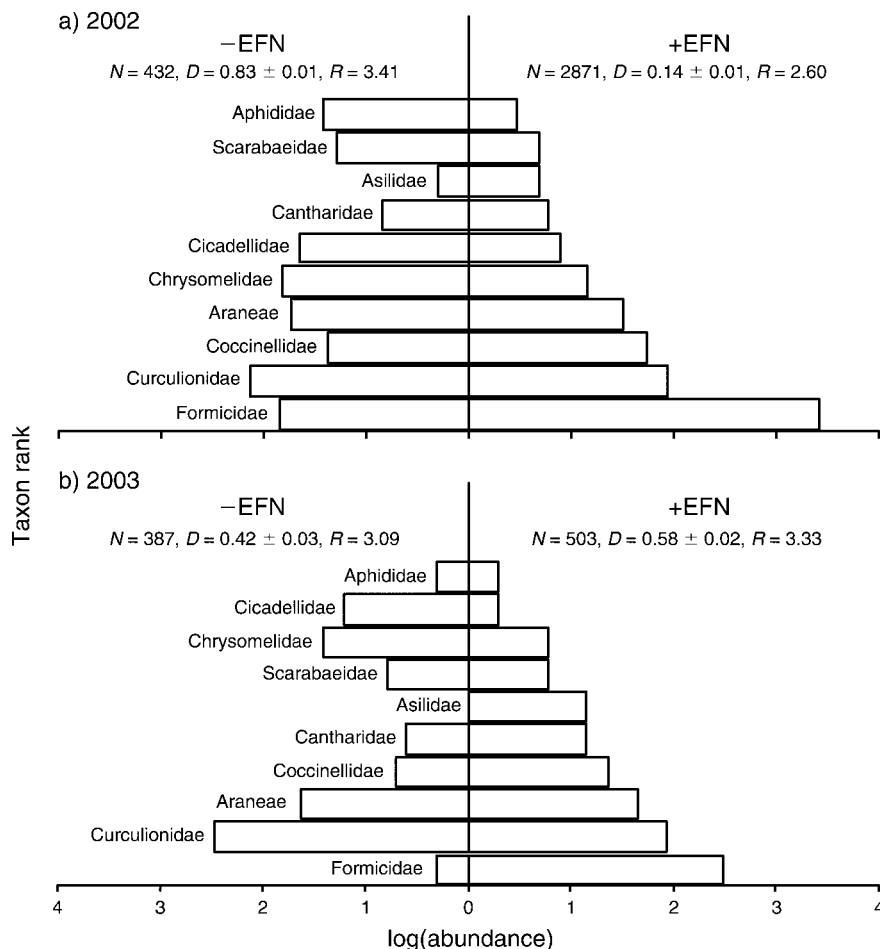


FIG. 1. Paired rank–abundance plots comparing the arthropod communities on peach trees with leaf extrafloral nectaries present (+EFN) or absent (–EFN). For each plot, the abundance of arthropods in each taxon on trees with EFNs is shown in rank order (right side), paired with the abundance of each corresponding taxon on trees lacking EFNs (left side). The total number of individuals ( $N$ ) and values calculated for Simpson's diversity index and standard error ( $D \pm SE$ ) and Margalef's richness index ( $R$ ) are shown for each tree type within years.

*Oedophrys hilleri* feeding consistently accounted for >98% of folivory observed on peach trees in the field, regardless of leaf EFN phenotype. A significant EFN effect on folivory was detected in July ( $F = 69.6$ ,  $df = 1, 3$ ,  $P = 0.004$ ) and August ( $F = 43.2$ ,  $df = 1, 3$ ,  $P = 0.007$ ). Trees with EFNs had less than one-half the level of folivory in July (injured leaves,  $32.0\% \pm 4.4\%$  vs.  $69.0\% \pm 4.4\%$ , mean  $\pm$  SE) and August ( $6.3\% \pm 4.6\%$  vs.  $36.3\% \pm 4.6\%$ ) than trees without EFNs (LSD,  $P < 0.05$ ).

**Tree productivity.**—EFNs significantly affected all three parameters of peach tree productivity (terminal C composition,  $F = 18.7$ ,  $df = 1, 3$ ,  $P = 0.02$ ; trunk diameter,  $F = 15.5$ ,  $df = 1, 3$ ,  $P = 0.03$ ; bud density,  $F = 12.1$ ,  $df = 1, 3$ ,  $P = 0.04$ ). After one year in the field, trees with EFNs had significantly more  $^{13}\text{C}$  (molar abundance,  $\Delta = 18.2 \pm 0.2$ , mean  $\pm$  SE) than trees without EFNs (molar abundance,  $\Delta = 18.0 \pm 0.0$ ; LSD,  $P < 0.05$ ). Average trunk diameter after two years of field

growth (October 2003) was significantly greater for trees with EFNs ( $46.5 \pm 1.9$  mm) than trees without EFNs ( $38.9 \pm 4.6$  mm; LSD,  $P < 0.05$ ). In the first year of fruit production (2004), significantly more flower buds were found on trees with EFNs than trees without EFNs (LSD,  $P < 0.05$ ; +EFN =  $7.4 \pm 1.0$  buds/terminal, –EFN =  $2.3 \pm 1.5$  buds/terminal, mean  $\pm$  SE).

#### The role of ants

**Ants' impact on arthropod community structure.**—The ant exclusion treatment successfully reduced ants on the EFN trees throughout the study months (Fig. 4a). Ants were consistently more abundant on EFN trees without the exclusion treatment (Fig. 4a). Ant abundance on the trees lacking EFNs was not affected by the ant exclusion treatment. The trees without EFNs harbored only one ant when the exclusion treatment was absent. Complete arthropod abundance data for trees with and without exclusion treatment are provided in Appendix D. When

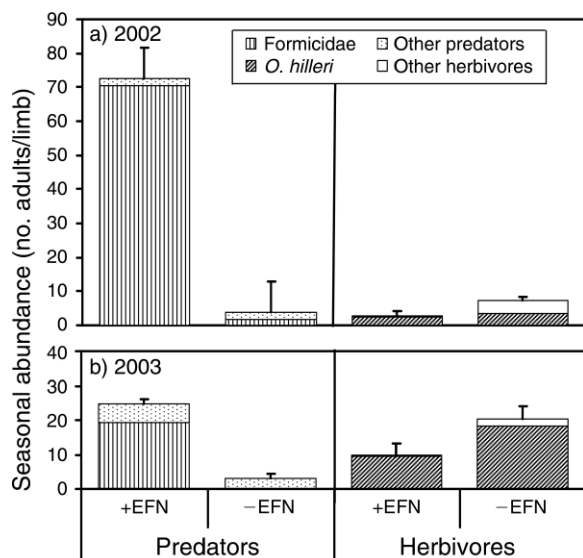


FIG. 2. Effect of peach tree leaf extrafloral nectary presence (+EFN) or absence (-EFN) on predator and herbivore abundances summed over 8 days in (a) 2002 and (b) 2003. Least-squares means (+SED) are shown. (SED is the standard error of difference.) "Other" predators include Araneae, Asilidae, Cantharidae, and Coccinellidae; "other" herbivores include Aphididae, Chrysomelidae, Cicadellidae, and Scarabaeidae.

not excluded, ants accounted for 55.7% of arthropods on EFN trees, compared to <1% on trees without EFNs (Appendix D). The herbivore *O. hilleri* consistently accounted for 75.5% of arthropods collected from trees

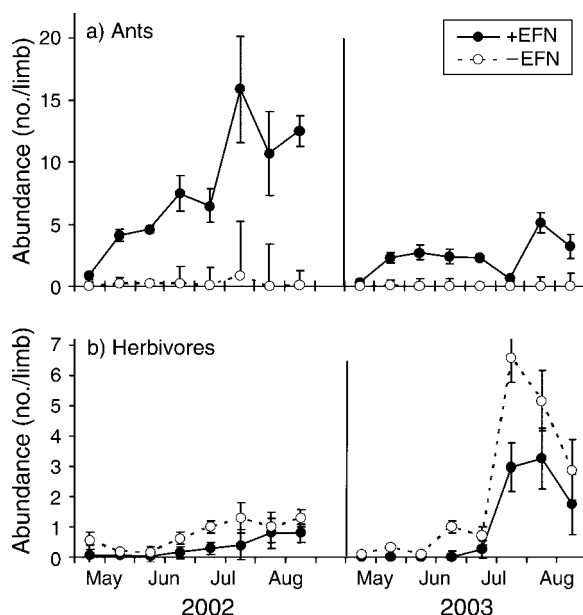


FIG. 3. Abundance (by limb jarring) of (a) ants and (b) herbivores on peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) in 2002 and 2003. Geometric means are plotted with 95% CI.

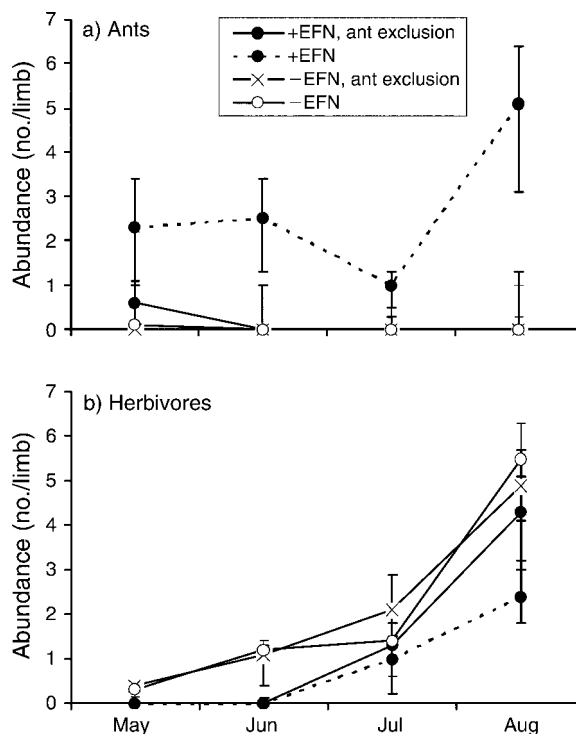


FIG. 4. Monthly abundance (by limb jarring) of (a) ants and (b) herbivores on peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) and with or without ant exclusion treatment, 2003. Geometric means with 95% CI are plotted. ANOVA revealed a significant EFN main effect (May and June) and ant exclusion effect (July) for herbivores.

without EFNs, regardless of the ant exclusion treatment. By contrast, when ants were excluded from the trees with EFNs, the weevil's contribution to the total arthropod load increased from 26.2% to 60.8%. Ant exclusion did not impact the structure of the overall arthropod community on trees of either leaf type (+EFN, control, diversity,  $0.61 \pm 0.018$ ,  $R = 3.29$ ,  $N = 542$ , mean  $\pm$  SE; +EFN, ant exclusion, diversity,  $0.60 \pm 0.030$ ,  $R = 3.68$ ,  $N = 278$ ; -EFN, control, diversity,  $0.42 \pm 0.030$ ,  $R = 3.09$ ,  $N = 387$ ; -EFN, ant exclusion, diversity,  $0.43 \pm 0.032$ ,  $R = 2.34$ ,  $N = 363$ ).

The ant exclusion treatment significantly affected herbivore abundance in July but not in other sample months (Appendix E). The overall herbivore load was significantly higher on trees when ants were excluded (LSD,  $P < 0.05$ ; Fig. 4b). A significant main effect of EFN phenotype was detected in May and June, when significantly more herbivores were collected from trees without EFNs (Appendix E; LSD,  $P < 0.05$ ; Fig. 4b). Neither ant exclusion nor EFN phenotype affected *O. hilleri* abundance when considered separately (ANOVA;  $P > 0.05$ ). The ant exclusion treatment significantly affected other (i.e., non-ant) predators in August ( $F = 8.1$ ,  $df = 1, 6$ ,  $P = 0.029$ ). Significantly more non-ant predators inhabited trees when ants were excluded during this period (LSD,  $P < 0.05$ ). The diversity and

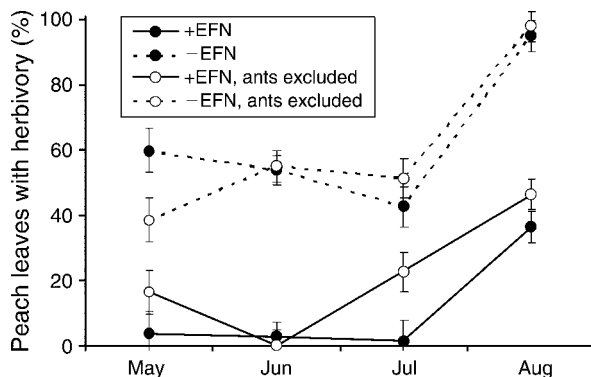


FIG. 5. Monthly folivory rates, measured as percentage of injured leaves in five leaf samples per tree in 2003, for peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) and with or without ant exclusion treatment: ANOVA revealed a significant interactive effect (May), ant exclusion effect (July), and EFN main effect (all months). Least-squares means ( $\pm$ SE) are shown.

richness of the predator group on trees with EFNs also increased when ants were excluded (control, diversity,  $0.38 \pm 0.001$ ,  $R = 1.55$ ,  $N = 388$ ; ant exclusion, diversity,  $0.71 \pm 0.001$ ,  $R = 2.01$ ,  $N = 98$ ).

**Ants' impact on folivory.**—A significant EFN by ant exclusion interaction was detected for folivory in May 2003 (Appendix E). When ants were present, a significant  $\sim 15$ -fold increase in folivory was observed for leaves without EFNs as compared to leaves with EFNs (LSD,  $P < 0.05$ ; Fig. 5). A significant EFN main effect was found for folivory during later months (June, July, and August; Appendix E). Leaves without EFNs had significantly higher folivory levels than leaves with EFNs during these months (LSD,  $P < 0.05$ ; Fig. 5). In July, the main effect of ant exclusion was also significant (Appendix E). The exclusion of ants from trees with EFNs resulted in a significant  $\sim 20$ -fold increase in folivory (LSD,  $df = 6$ ,  $P = 0.01$ ; Fig. 5). *O. hilleri* and leaf herbivory were significantly correlated on both tree types when ants were excluded (+EFN,  $\rho = 0.73$ ,  $P = 0.001$ ; -EFN,  $\rho = 0.51$ ,  $P = 0.039$ ; Spearman's rank correlation,  $N = 16$ ).

#### DISCUSSION

This study revealed that EFNs can significantly alter arthropod community structure and benefit peach trees, particularly during the first year of seedling establishment. Ants dominated the arthropod community on trees with EFNs during the colonization phase (four months after planting), significantly outnumbering herbivores and other predators. The situation was considerably different on trees without EFNs, where herbivores outnumbered the ants and other predators. The dominance of ants could be crucial to plant establishment in the first year of growth, when the seedlings are more vulnerable to herbivory. The most prevalent and most injurious folivore, *O. hilleri*, was much less abundant on trees with EFNs than trees without EFNs during seedling establishment. Protection during seedling establishment is expected to have particular evolutionary benefits because survival to the reproductive age (i.e., "recruitment") is a requisite for genotype dissemination.

EFN production would theoretically be costly to the trees if they attracted more herbivores and subsequently suffered more folivory or were less productive than the trees without EFNs. Our results indicate that because herbivores were less prevalent on the trees with EFNs (Fig. 3b), the trees benefited from  $>50\%$  less folivory than trees without EFNs. Both terminal carbon assimilation ( $^{13}\text{C}$ ) and trunk growth were higher for trees producing EFNs. These growth enhancements apparently carried over to the initial fruiting period, when the trees with EFNs had a threefold increase in bud production compared to trees without EFNs. Therefore, any potential metabolic costs associated with producing the EFN structures and secretions were apparently outweighed by the growth advantages conferred by the EFN-mediated protection from folivory. These findings are consistent with the "protectionist" hypothesis involving EFNs and ant mutualists that has been supported in numerous other systems (Bronstein 1998).

If the EFN-mediated plant defense revealed in our first study was exclusive to ants as a functional group, then we would expect to see an increase in folivory when ants were excluded from the trees with EFNs. Alternatively, if a complex of predators collectively benefited the tree, then it would be expected to maintain a significant benefit from EFNs following ant exclusion. The results of our second study indicate that ant exclusion from the trees with EFNs triggered a shift that favored the chief defoliator, *O. hilleri*, which became the dominant arthropod on those trees. The defoliator accounted for 60.8% of arthropods on trees with EFNs when ants were excluded, compared to 26.2% without ant exclusion (Appendix D). Nectar production patterns may be synchronized with the most susceptible stages of plant growth (Bentley 1977, Wäckers and Bonifay 2004) or with the times during which damaging herbivores are typically active (Tilman 1978). We found that folivory for trees with EFNs increased  $>5\times$  if ants were excluded in May, a time when the trees bear young leaf material that is not chemically protected (Fig. 5). Although the exclusion of ants did not significantly affect *O. hilleri* densities within each sample month, the defoliator was positively correlated with levels of leaf injury only when

ants were excluded. This suggests that ants successfully deterred or disrupted the *O. hilleri* from feeding, although the exact mechanism is not known. Folivory rates were consistently lower for trees with EFNs than for trees without EFNs in every month (Fig. 5). However, protection cannot be attributed to ants in August, when ant exclusion had no effect on folivory for the EFN trees (Appendix E). That significantly more non-ant predators were present on the EFN trees with ants excluded in August suggests that other (non-ant) predators can assume the EFN-mediated protective role in the absence of ants. The availability of EFN resources for other (non-ant) predators apparently leads to some degree of functional redundancy with regard to the protective mechanism.

Our results provide direct evidence that ants are consistently more prevalent on peach trees that produce EFNs and that the EFNs on young trees mediate protection from folivory with a resulting increase in plant productivity in later stages. Future selections of agricultural peach cultivars therefore should seek to retain the EFN trait, although these findings may or may not apply to orchards with different ant species. Some peach cultivars without EFNs already have been produced inadvertently through modern fruit breeding programs (Okie 1998). A shift to EFN-less peach cultivars could seriously undermine current efforts to develop ecologically based orchard systems that reduce insecticide inputs and conserve naturally occurring predators. Our findings also demonstrate that EFN-mediated protection from folivores is not always exclusive to ant mutualists, emphasizing the need to consider effects on the whole arthropod community, rather than a single predator taxon, in the study of resource-mediated plant defenses. A broader approach may be particularly important when researchers aim to apply findings to our understanding of naturally occurring pest regulation or the enhancement of natural enemies via provision of resources (e.g., conservation biological control).

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#### LITERATURE CITED

- Bentley, B. L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* 8:407–427.
- Bronstein, J. L. 1998. The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* 30: 150–161.
- Caldwell, D. L., and K. O. Gerhardt. 1986. Chemical analysis of peach extrafloral nectary exudate. *Phytochemist* 25:411–413.
- Connors, C. H. 1922. Inheritance of foliar glands of the peach. *Proceedings of the American Society of Horticultural Science* 18:20–26.
- Cuautle, M., and V. Rico-Gray. 2003. The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Functional Ecology* 17:417–423.
- Ehleringer, J. R. 1991.  $^{13}\text{C}/^{12}\text{C}$  fractionation and its utility in terrestrial plant studies. Pages 187–200 in D. C. Coleman and B. Fry, editors. *Carbon isotope techniques*. Academic Press, San Diego, California, USA.
- Gregory, C. T. 1915. The taxonomic value and structure of the peach leaf glands. *Cornell University Agricultural Experiment Station Bulletin Number 365*. Ithaca, New York, USA.
- Heil, M., A. Hilpert, R. Kruger, and K. E. Linsenmair. 2004. Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. *Journal of Tropical Ecology* 20:201–208.
- Lingren, P. D., and M. J. Lukefahr. 1977. Effects of nectariless cotton on caged populations of *Campoplex sonorensis*. *Environmental Entomology* 6:586–588.
- Longino, J. T., and R. K. Colwell. 1997. Biodiversity assessment using structured inventory: capturing the ant fauna of a tropical rain forest. *Ecological Applications* 7: 1263–1277.
- Margalef, R. 1958. Information theory in ecology. *General Systematics* 3:36–71.
- Mathews, C. R. 2005. Role of peach [*Prunus persica* (L.) Batsch] extrafloral nectaries in mediating natural enemy–herbivore interactions. Dissertation. University of Maryland, College Park, Maryland, USA.
- Mathews, C. R., M. W. Brown, and D. G. Bottrell. 2007. Leaf extrafloral nectaries enhance biological control of a key economic pest, *Grapholita molesta* (Lepidoptera: Tortricidae), in peach (Rosales: Rosaceae). *Environmental Entomology* 36:383–389.
- McKey, D. 1974. Adaptive patterns in alkaloid physiology. *American Naturalist* 108:305–320.
- Mody, K., and K. E. Linsenmair. 2004. Plant-attracted ants affect arthropod community structure but not necessarily herbivory. *Ecological Entomology* 29:217–225.
- Okie, W. R. 1998. Handbook of peach and nectarine varieties. USDA Agricultural Research Service, Agriculture Handbook Number 714. Byron, Georgia, USA.
- Pemberton, R. W. 1993. Observations of extrafloral nectar feeding by predaceous and fungivorous mites. *Proceedings of the Entomological Society of Washington* 95:642–643.
- Pemberton, R. W., and N. J. Vandenberg. 1993. Extrafloral nectar feeding by ladybird beetles (Coleoptera: Coccinellidae). *Proceedings of the Entomological Society of Washington* 95:139–151.
- Putman, W. L. 1963. Nectar of peach leaf glands as insect food. *Canadian Entomologist* 95:108–109.
- Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. Pages 4–53 in B. A. Rosenthal and H. D. Janzen, editors. *Herbivores: their interaction with secondary plant metabolites*. Academic Press, London, UK.
- Rogers, C. E. 1985. Extrafloral nectar: entomological implications. *Bulletin of the Entomological Society of America* 31: 15–20.
- Ruhren, S., and S. Handel. 1999. Jumping spiders (Salticidae) enhance the seed productions of a plant with extrafloral nectaries. *Oecologia* 119:227–230.
- SAS Institute. 1999. SAS system, release 8.02. SAS Institute, Cary, North Carolina, USA.
- Simpson, E. H. 1949. Measurement of diversity. *Nature* 163:688.
- Southwood, T. R. E. 1966. *Ecological methods with particular reference to the study of insect populations*. Chapman and Hall, London, UK.



- Tilman, D. 1978. Cherries, ants and tent caterpillars: timing of nectar production in relation to susceptibility of caterpillars to ant predation. *Ecology* 58:686–692.
- Wäckers, F. L., and C. Bonifay. 2004. How to be sweet? Extrafloral nectar allocation in *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology* 85:1512–1518.
- Wäckers, F. L., J. Romeis, and P. C. J. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. *Annual Review of Entomology* 52: 301–323.
- Weber, C. A., W. B. Sherman, and G. A. Moore. 1997. Linkage and correlation analysis of some traits in peach. *Fruit Varieties Journal* 51:246–250.
- Westwood, M. N., and A. N. Roberts. 1970. The relationship between trunk cross-sectional area and weight of apple trees. *Journal of the American Society for Horticultural Science* 95: 28–30.
- Yokoyama, V. Y., and G. T. Miller. 1989. Stone fruit foliar nitrogen and extrafloral nectar in relation to cigarette beetle (Coleoptera: Anobiidae) growth on the leaves. *Journal of Economic Entomology* 82:1675–1678.

#### APPENDIX A

Adult arthropod abundance (by limb jarring) on peach trees with and without extrafloral nectaries (+EFN, –EFN), by year (*Ecological Archives* A019-030-A1).

#### APPENDIX B

Results of mixed-model ANOVAs testing for fixed effect of leaf EFNs on annual arthropod predator and herbivore densities on peach trees in a completely randomized design field study (*Ecological Archives* A019-030-A2).

#### APPENDIX C

Results of mixed-model ANOVAs testing for fixed effect of leaf EFNs on ant and herbivore abundances on peach trees (*Ecological Archives* A019-030-A3).

#### APPENDIX D

Adult arthropod abundance (by limb jarring) on peach trees with and without extrafloral nectaries (+EFN, –EFN) and with and without “control” ant exclusion in 2003 (*Ecological Archives* A019-030-A4).

#### APPENDIX E

Results of ANOVAs testing for fixed effects of leaf EFNs, ant exclusion treatment, and interaction on herbivore abundances and folivory for peach trees in CRD split-plot field study; sampling was performed monthly (May–August) in 2003 (*Ecological Archives* A019-030-A5).